

# Murine models of genetic susceptibility to beryllium

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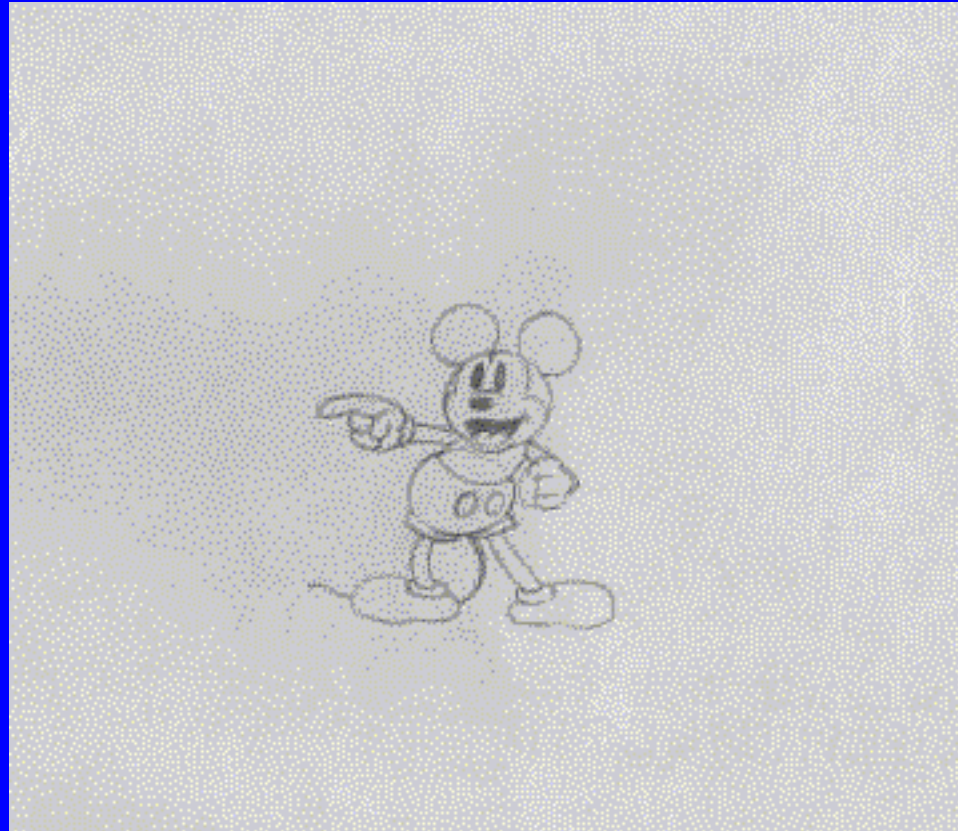
(NIEHS)

# Research Approach

- Like many other lung diseases (e.g., asthma, COPD), CBD has a polygenic inheritance.
- Although we know that *HLA-DPB1* plays a role, there are likely other modifier genes.

# Research Approach

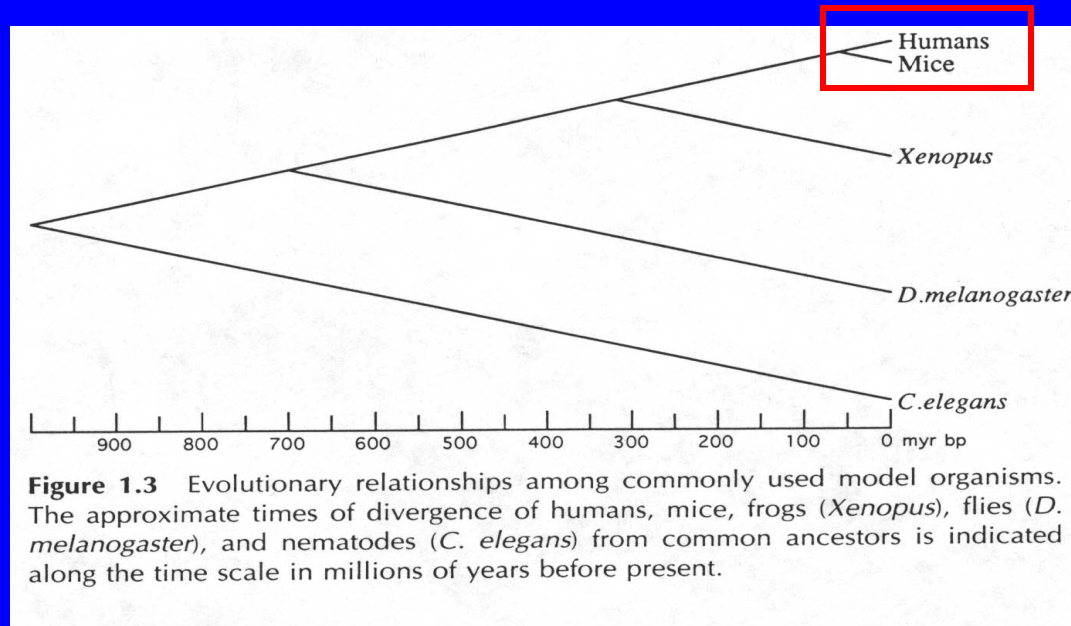
1. Examine differential response to beryllium in genetically defined inbred and transgenic mouse strains to uncover candidate genes that may be responsible for or modify CBD
  - Mouse ear-swelling test
    - Looks at the sensitization process
  - Oropharyngeal aspiration study
    - Focuses on granuloma formation
2. Compare beryllium metal vs. oxide particles
3. Identify better biomarkers of sensitization and CBD

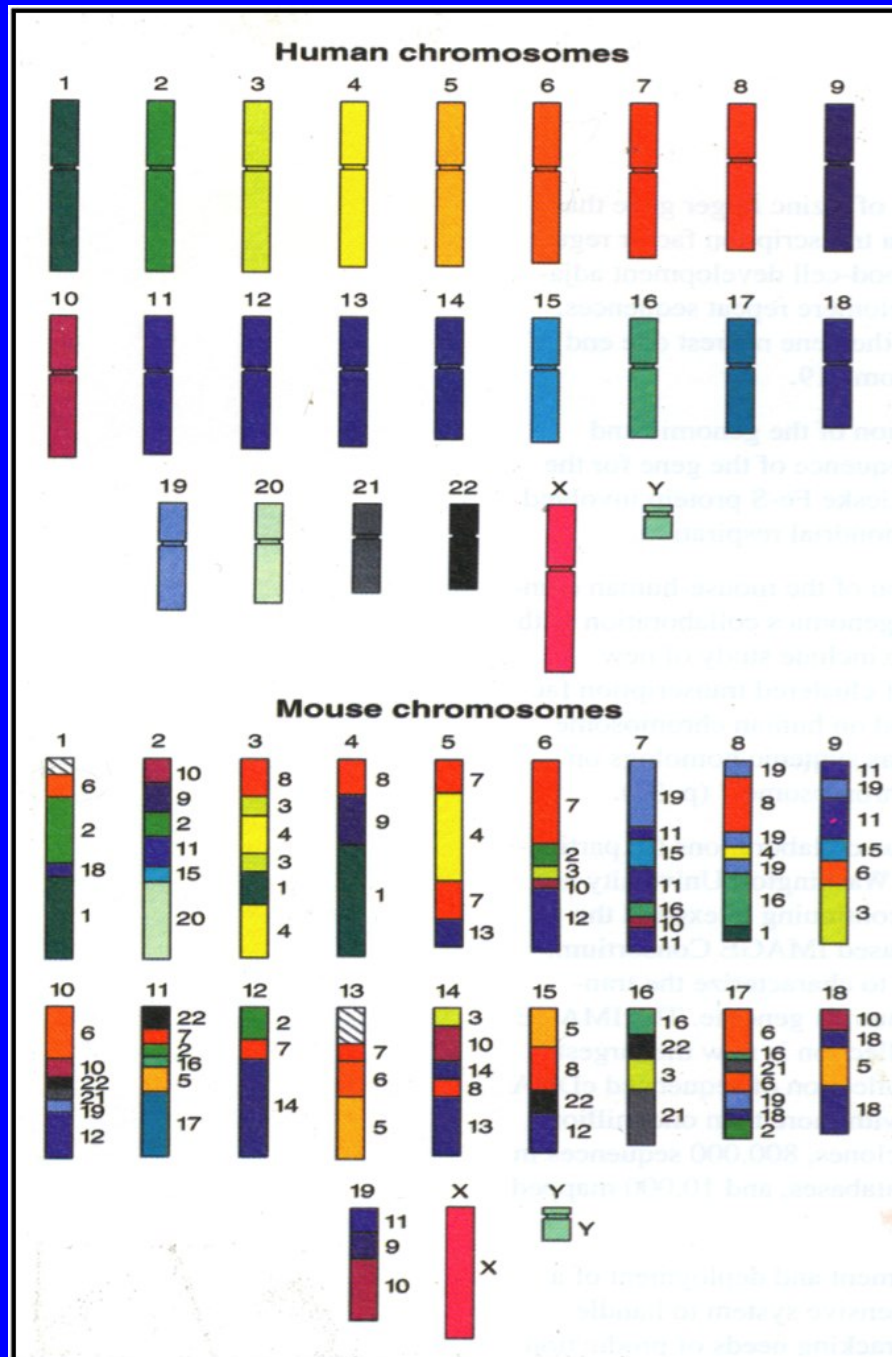




## *Most Important Reason to Use Mice for Genetic Studies?*

Due to their evolutionary relationship, inbred mice share several chromosomal regions of conserved synteny with humans.





## *Genetic homology of human and mouse genomes*

- Colors and corresponding numbers on the mouse chromosomes indicate the human chromosomes containing homologous segments
- D.O.E. Human Genome Program Report, 1997

# Approaches to Study the Role of Genetic Susceptibility in Lung Disease in Mice

- 3 approaches:
  - Have evidence for or guess specifically involved gene(s)
    - Transgenic or knockout mice
    - H-2 models
  - Classic genetic mouse models
    - Linkage studies - Kleeberger, Leikauf, and Schwartz
  - Computational methods
    - Haplotype mapping - Peltz and Wiltshire



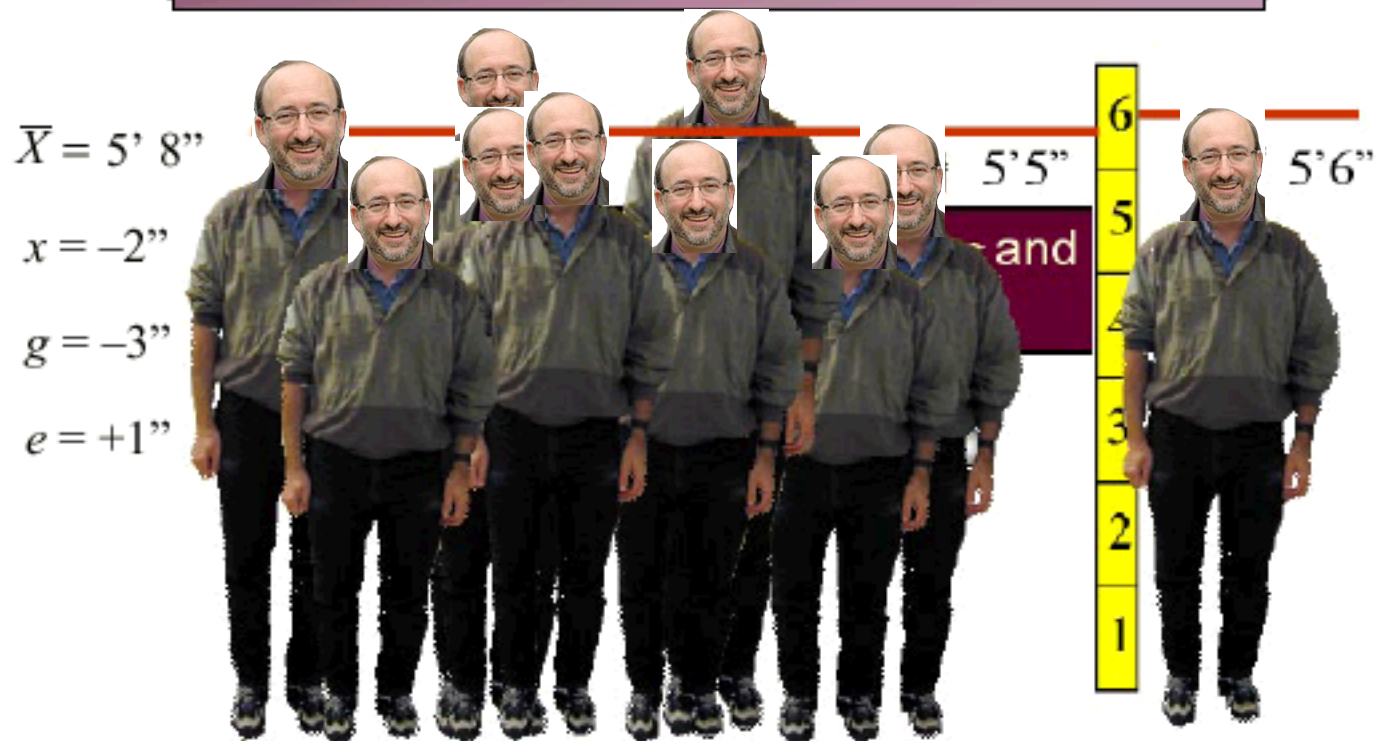
*If you're going to use animals, what do you need to find a gene(s) responsible for susceptibility?*

- Groups of animals which are genetically homogeneous within a group and heterogeneous among groups
- These groups of animals must show differences in response (i.e., phenotype) and these differences must be quantitative

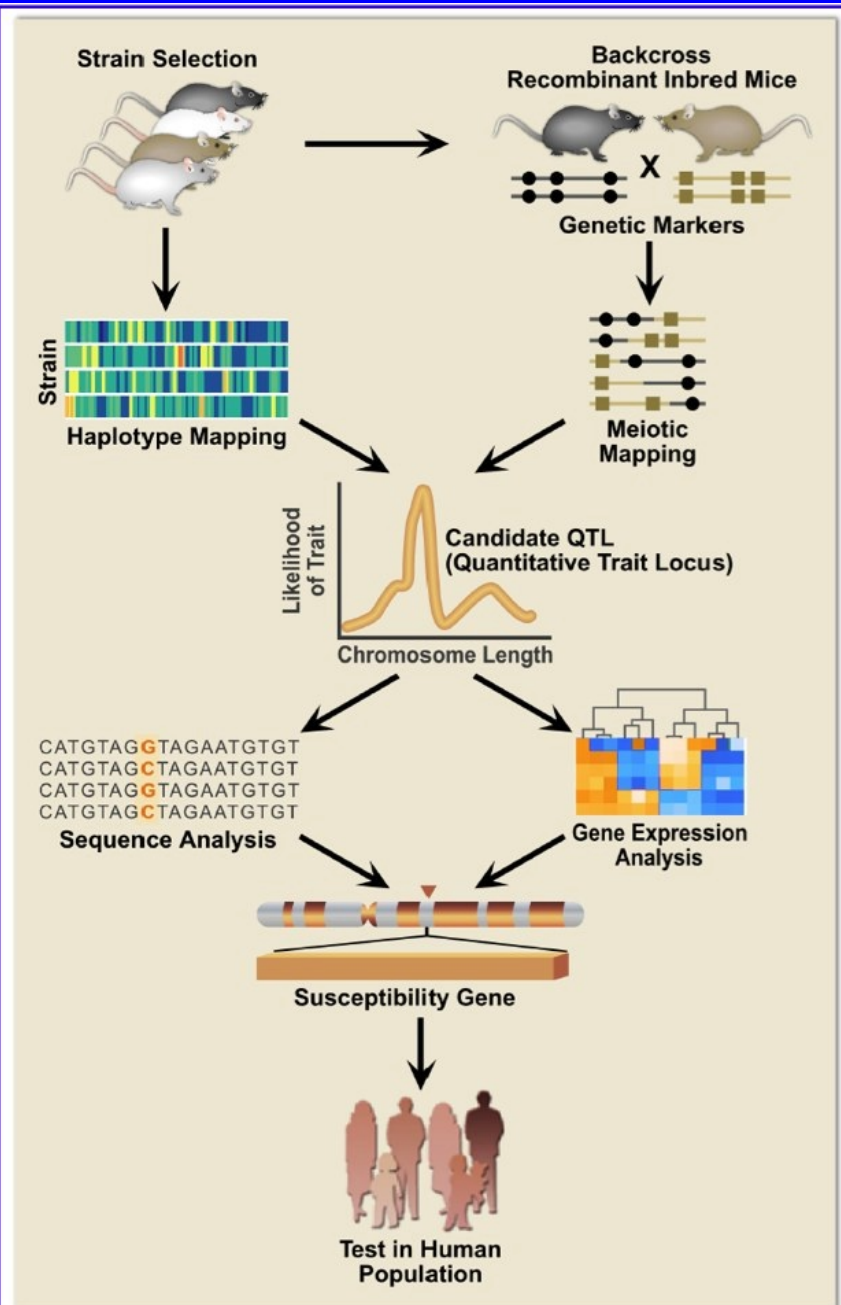


# QUANTITATIVE GENETICS

*What is relative importance of genes versus environment? ("nature vs. nurture")*



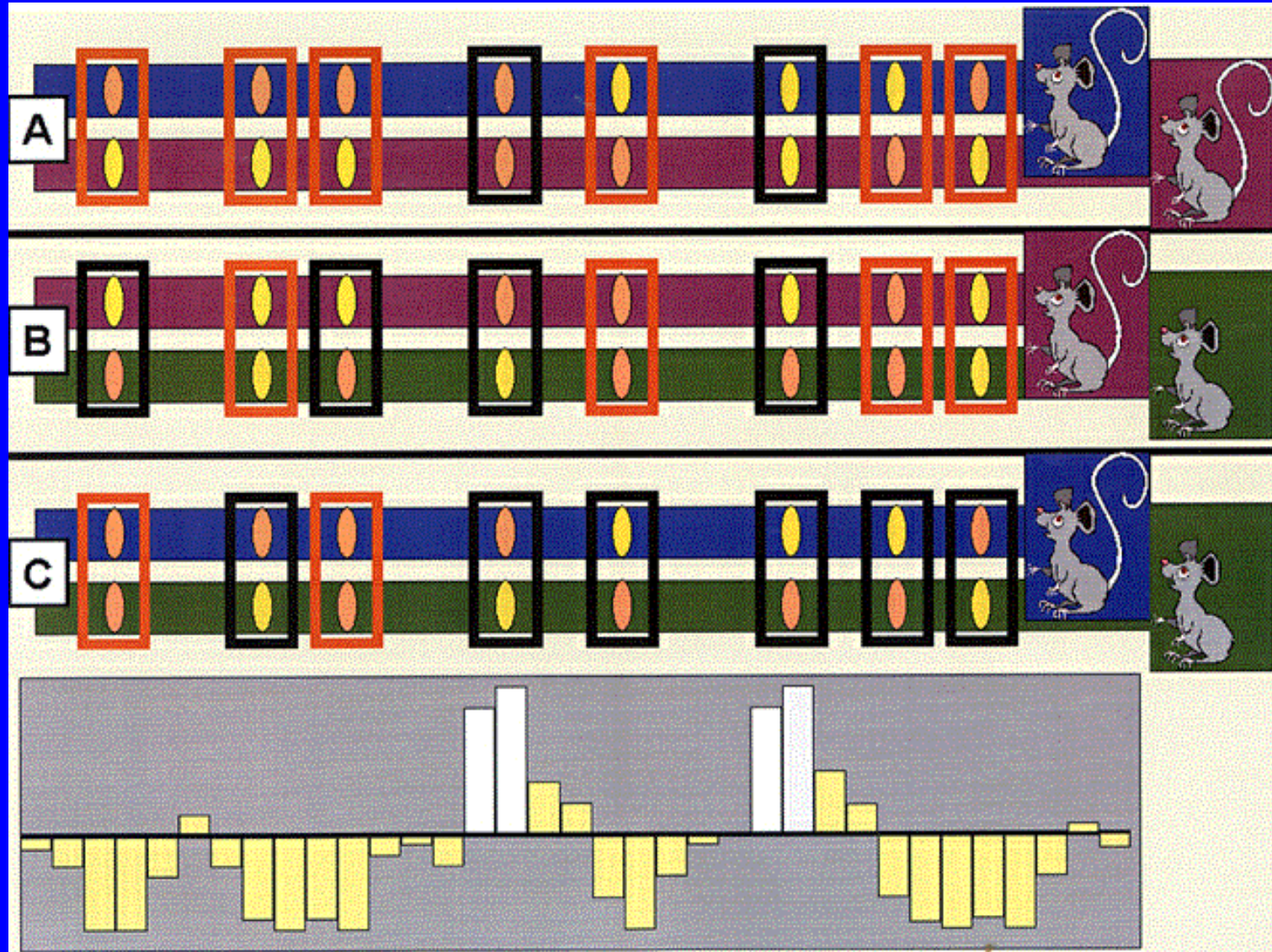
## Schematic Representation of Positional Cloning Strategy



Adapted from Cho & Kleeberger. "Genetic Mechanisms of Susceptibility to Oxidative Lung Injury in Mice" *Free Radical Biology and Medicine* 42:433-445, 2007.



# Diagrammatic representation of the computational prediction method





# Beryllium - Genetic Susceptibility



# Evidence for Genetic Factors in Granulomas

- Human clinical studies with CBD
  - Obvious evidence of genetic susceptibility factors
- Animal studies using inbred strains
  - pigeon dropping extract in mice
  - guinea pig - beryllium
  - mouse strain studies
    - ♦ Finch/Nikula/Benson/Hoover
    - ♦ Gordon

# Approaches to Study Role of Genetic Susceptibility Factors in Granulomatous Lung Disease

- 3 approaches
  - Have evidence for or guess specifically involved genes
    - Transgenic or knockout mice - *Tinkle/Gordon*
    - H-2 models - *Benson*
  - Classic genetic mouse models
    - Linkage studies
  - Computational methods - *Gordon*

# What We Knew from Animal Studies

- Single large dose of beryllium leads to granulomas in:
  - Monkeys, dogs, guinea pigs, and mice but not rats
- Granulomatous changes are similar to human CBD histologically
- Granulomas don't seem to be progressive and evidence for regression

# Lovelace Mouse Studies

- Strain - A/J, C3H/HeJ mice
- Dose - single inhalation exposure
  - Body burden 1.7 to 64  $\mu\text{g}$
- Produced granulomas – no strain differences

# 2nd Lovelace Strain Response Study

- Hypothesized:
  - Be-induced pulmonary lesions is a cell-mediated immune response and the magnitude of response is influenced by the MHC II complex (H-2 allele)

# Results

- Ranking for immune and inflammatory response:
  - C3H/HeJ > C57BL/6, B10.A (4R) > A<sup>b</sup>beta
- H-2 allele is important in the magnitude of immune response to Be in mice
  - haplotype k predominates

# Strain Response Studies at NYU

- No pre-conceived notion of involved genes
- Hypothesized: CBD in mice is a complex trait with multiple genes involved

# NYU Experiments

- Skin sensitization
  - transgenic mice
  - inbred strains
- Repeated lung aspiration with beryllium metal and oxide
- Skin sensitization preceding lung aspiration - *future*



# Transgenic Mouse Hypothesis

- Insertion of the antigen presenting moiety, HLA-DPB1 Glu 69, into an animal genome would provide correct beryllium presentation to T cells and subsequent pulmonary granuloma formation
  - Tinkle, Rubin, Wu, Weston, Hubbs, Hoover

# Transgenic Mice

- HLA-DPB1\*0401 (low susceptibility)
- HLA-DPB1\*0201 (increased susceptibility)
- HLA-DPB1\*1701 (hyper-susceptibility)

# Mouse Ear Swelling Test (MEST)

## Timeline

Day 0:	Day 1 – 3:	Day 4 – 7:	Day 8:	Day 9:	Day 10 – 12:
Shave mouse back	Sensitize mouse on back	Rest	Baseline Measurement and challenge on ear	Measurement and challenge on ear	Measurement of ear

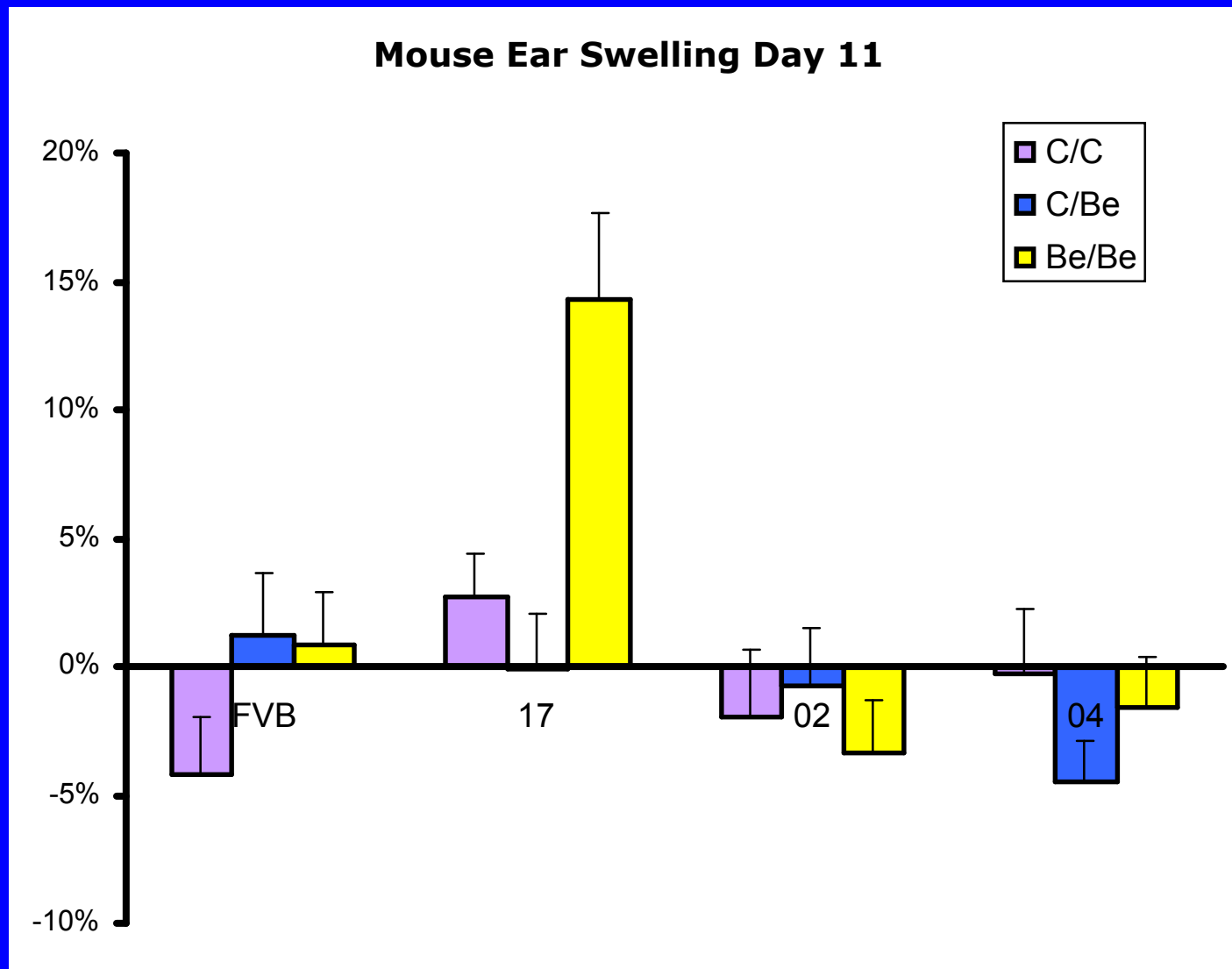
## Measurements

- End-point measured is swelling of right ear
- Average of two readings using digital micrometer
- Reported as percentage of difference versus the day 8 baseline (thickness of the right ear of each mouse before the challenge)

# Ear Swelling Test

<u>Group</u>	<u>Sensitization</u>	<u>Challenge</u>
<b>Control/Control (C/C)</b>	water/phthalate	water/phthalate
<b>Control/Beryllium (C/Be)</b>	water/phthalate	1M BeSo <sub>4</sub> /phthalate
<b>Beryllium/Beryllium (Be/Be)</b>	1M BeSo <sub>4</sub> /phthalate	1M BeSo <sub>4</sub> /phthalate

# Transgenic mice ear swelling results



# Transgenic mice ear swelling results

- Response of mice with human transgenes for susceptibility came out as predicted
  - Hyper-susceptible \*1701 mice had a significantly greater skin sensitization response
- Good model for studying modifier genes in sensitization portion of CBD and for granuloma formation

# Inbred Mice – Ear Swelling Study

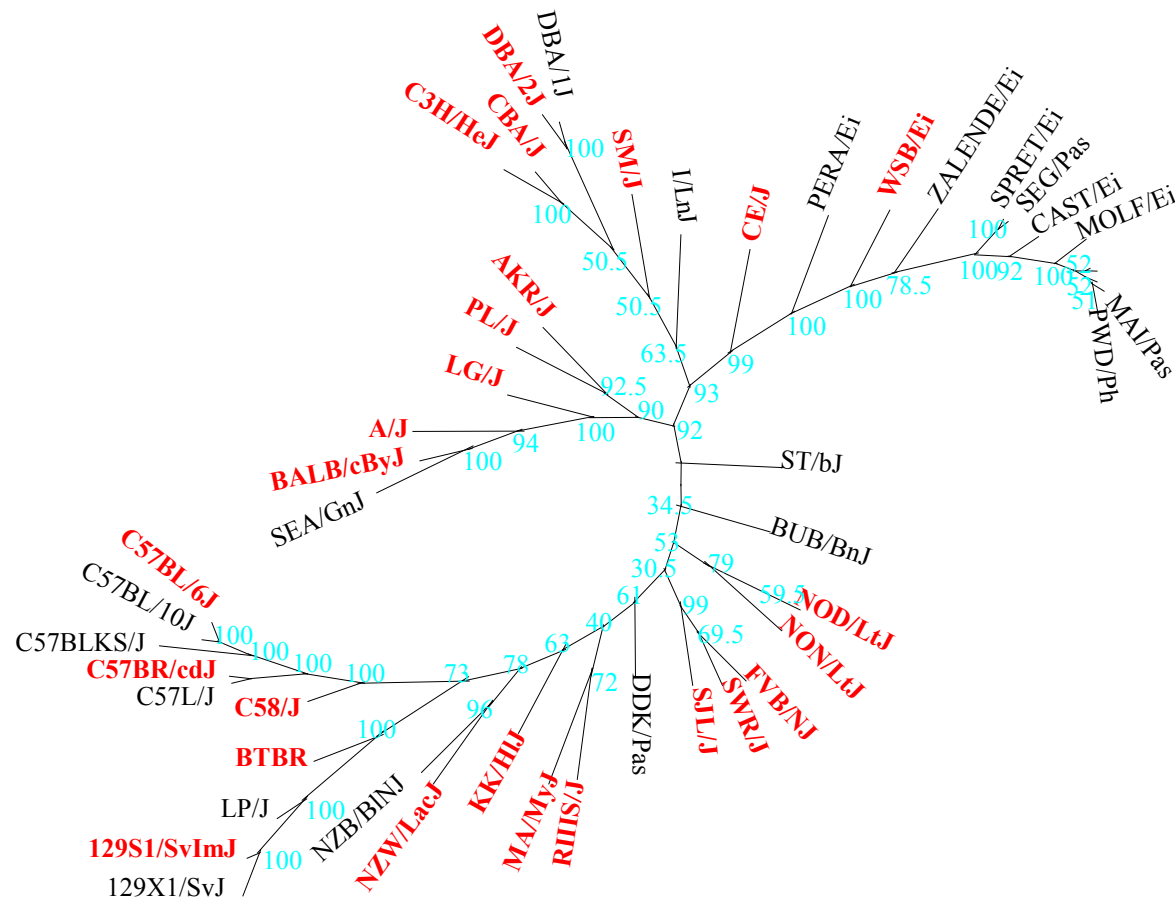
Classic intercross genetic experiment?

or

*In silico* experiment?

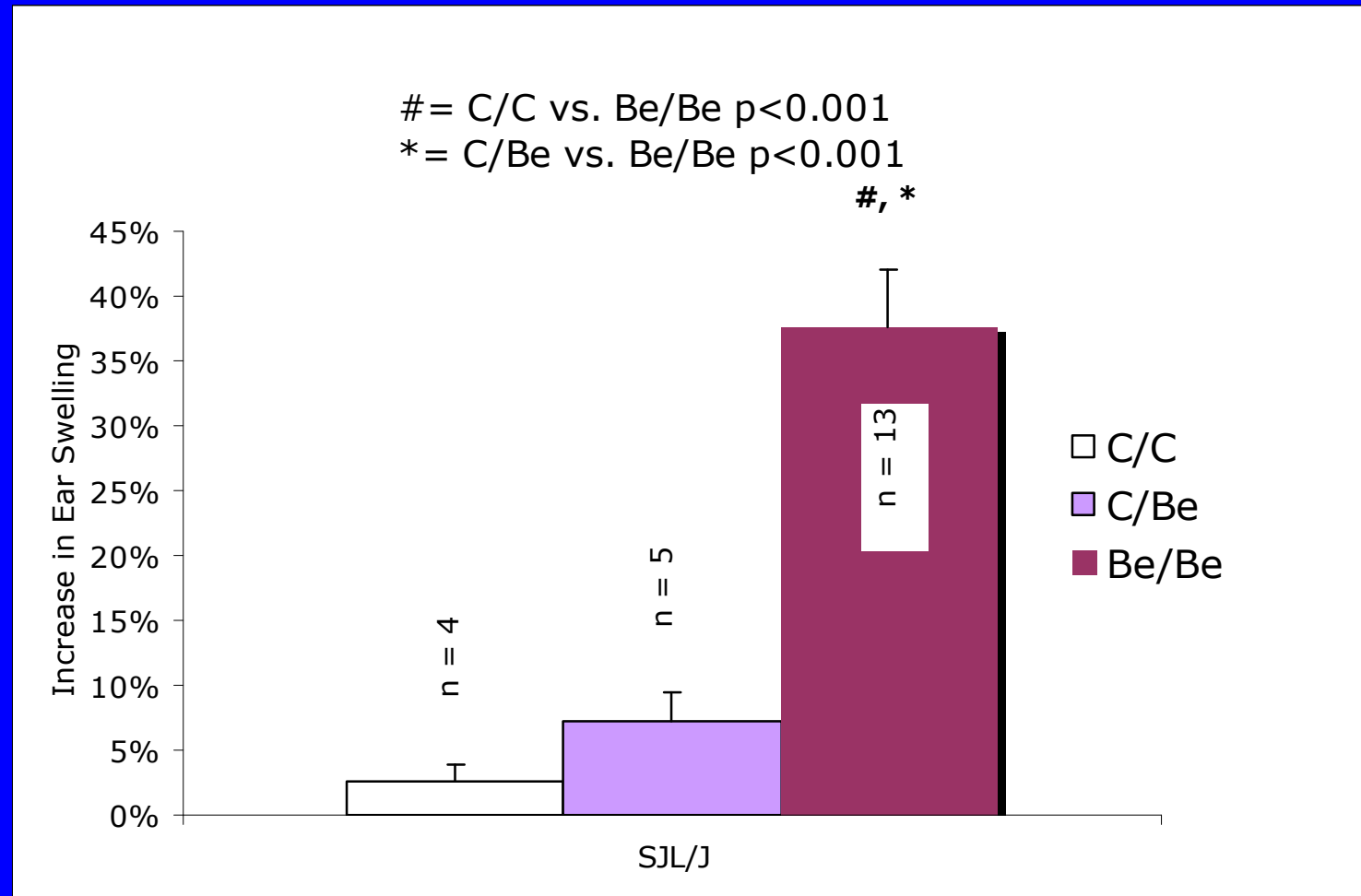
# *In silico* experiment

## Mouse Phylogenetic Tree



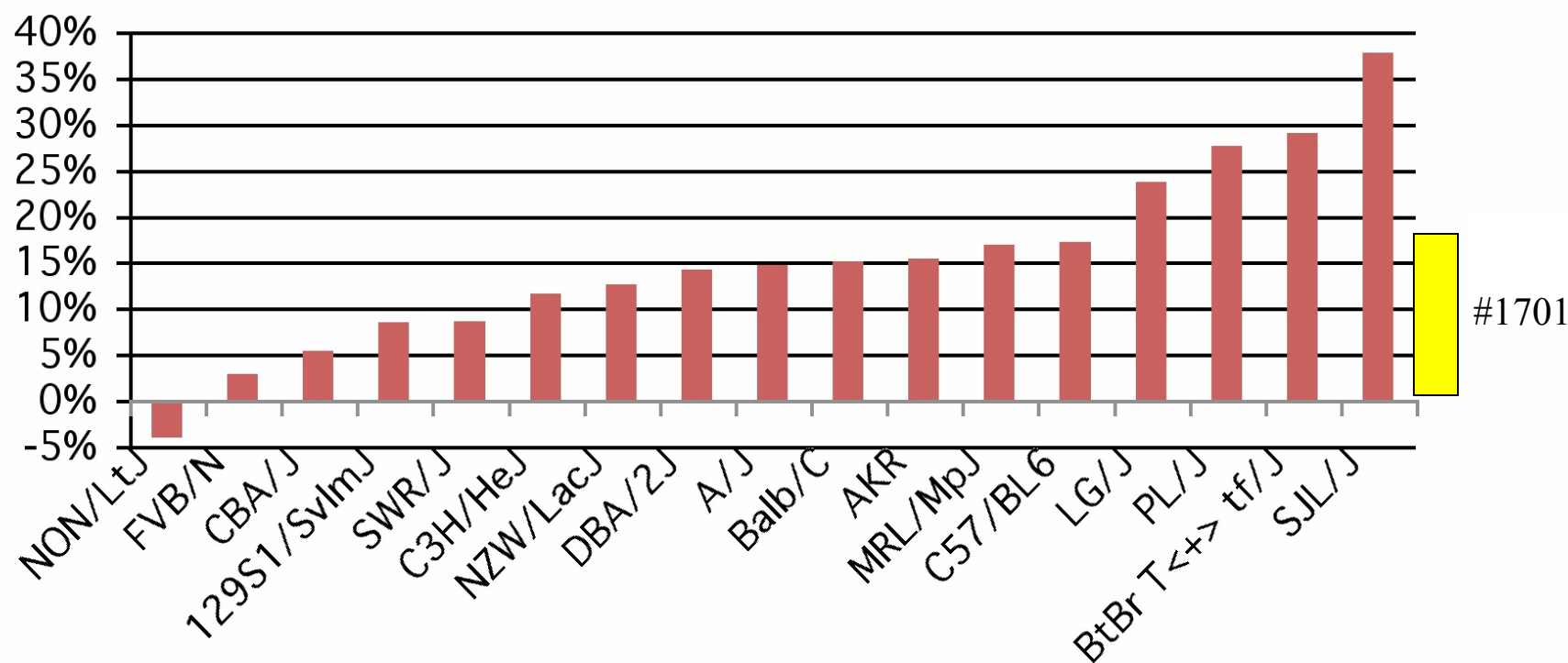


# Was ear swelling due to irritation or a true immune response to beryllium?

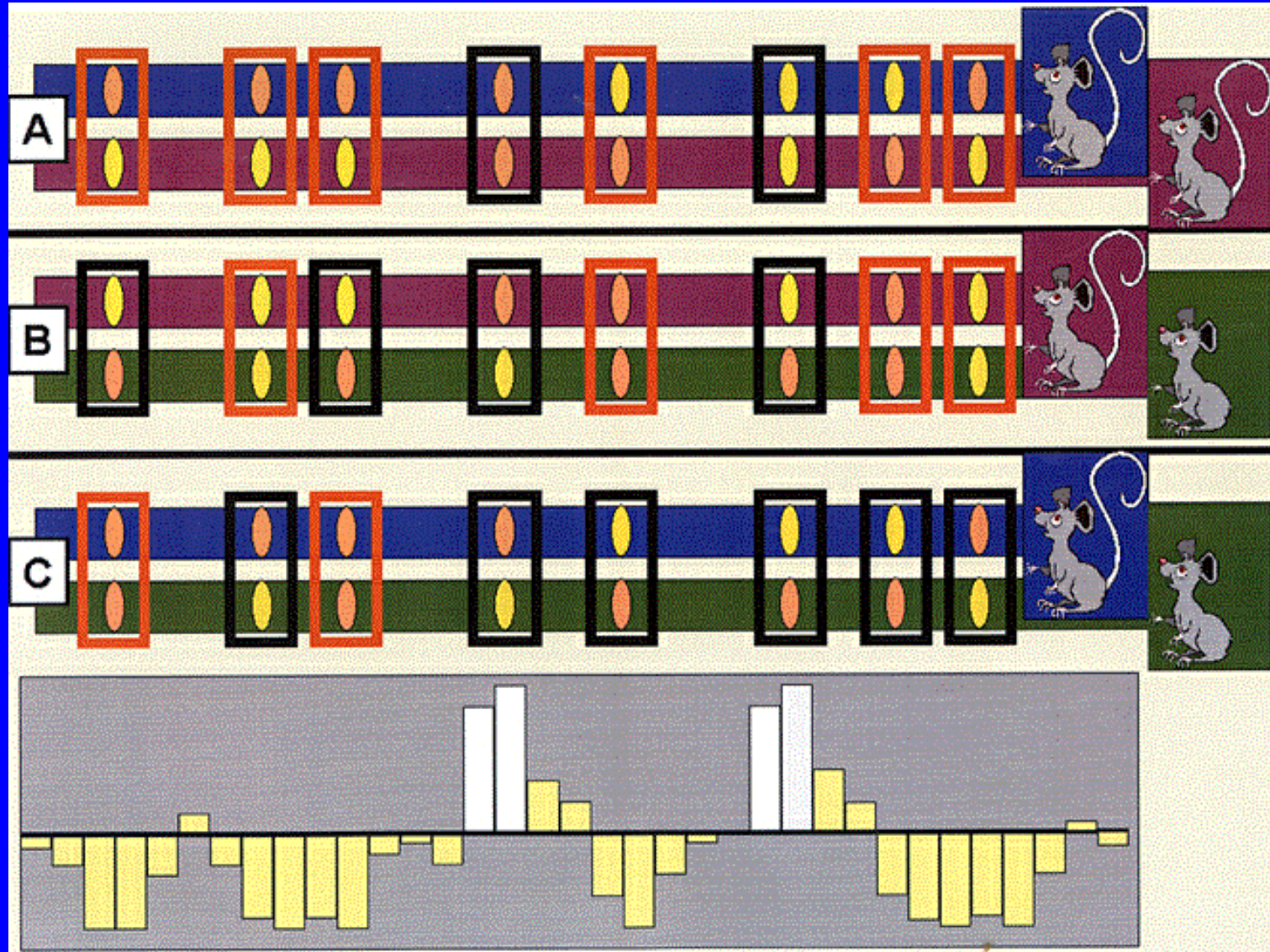


## Differential Response in Ear Swelling to Beryllium Sensitization and Challenge in 17 inbred mouse strains

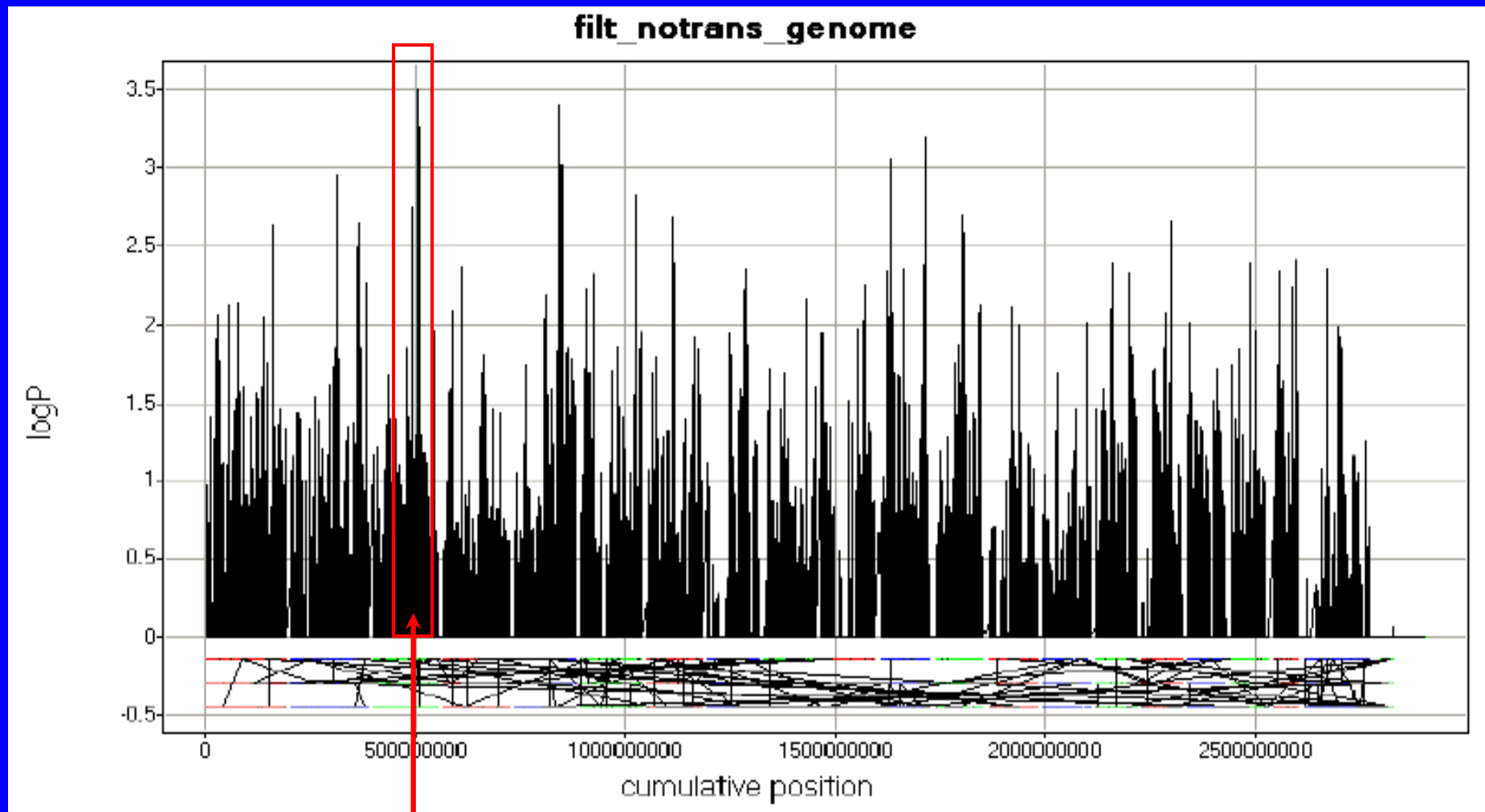
### Mouse Ear Swelling Test Max Day



# Diagrammatic representation of the computational prediction method

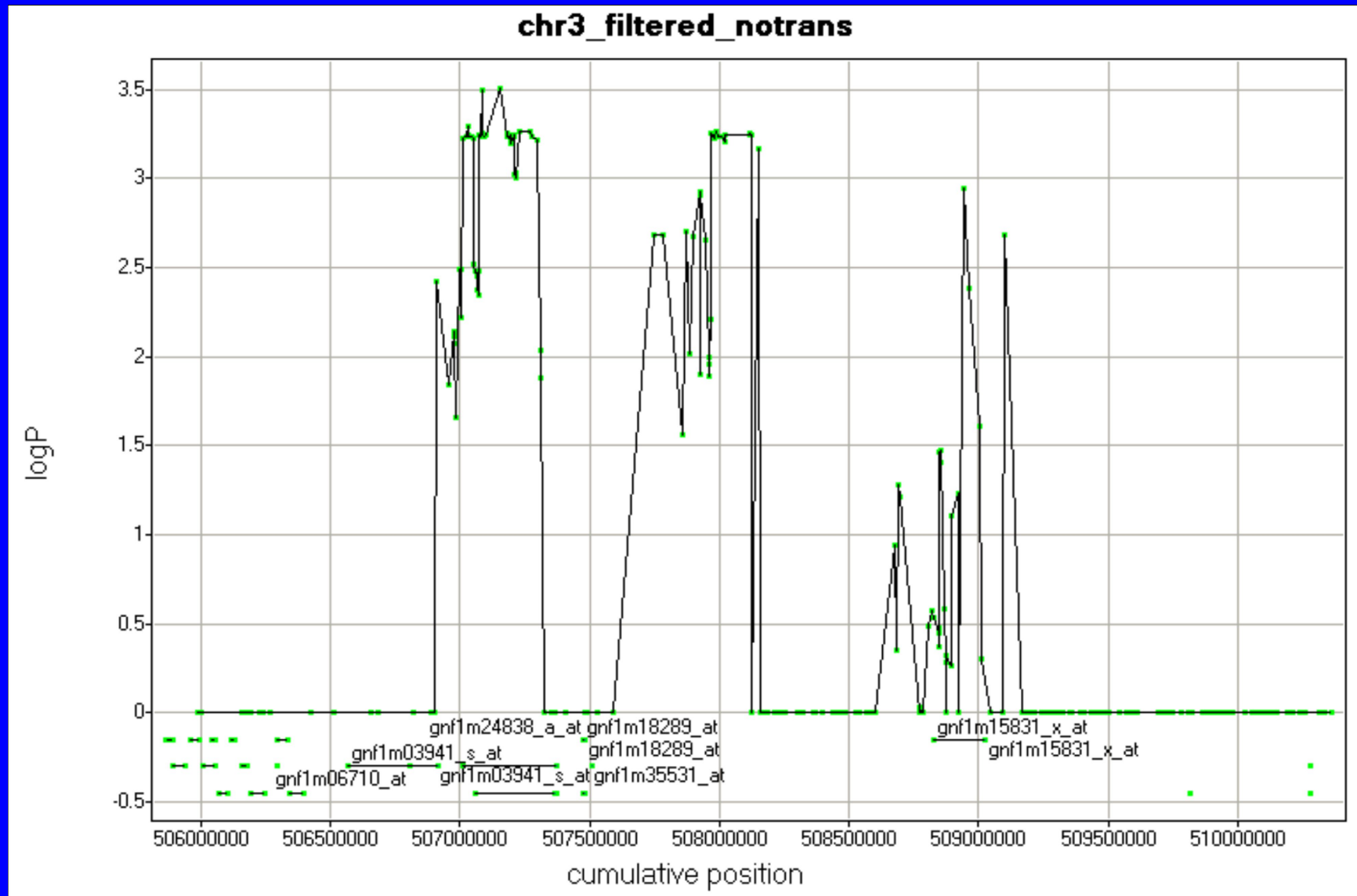


# Haplotype Mapping of Sensitization



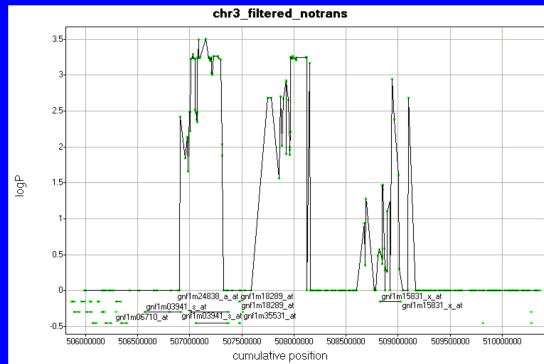
Chr. 3

# Haplotype Mapping of Sensitization





# Haplotype Mapping of Sensitization



- Vav family of Rho guanine nucleotide exchange factors is thought to orchestrate signaling events downstream of lymphocyte antigen receptors
- Vav3 plays a critical compensatory function in T cells and there is an essential role for the entire Vav protein family in lymphocyte development and activation.

# Granuloma Methods (Round One)

- 5 strains of inbred mice
  - BALB/c, C3H/HeJ, A/HeJ, 129/Sv, and C57BL/6
- 25  $\mu\text{g}$ /mouse, once/month for 4 months

# Results - Repeated Instillations

- Strain differences in response

- $A/HeJ < 129 < C3H/HeJ = C57BL/6 < BALB/c$



# Conclusion

- A good model for examining genetic factors contributing to beryllium-induced lung granulomas
- Repeated the study with more inbred strains (Round 2), but found no response

# Repeated the Strain Response Study (Round 3)

- 7 inbred strains
  - Aspiration with 20, 35, or 50  $\mu\text{g}$  of fresh beryllium metal particles or water vehicle monthly
  - Strains: A/J, Balb/c, C3H/HeJ, C57BL/6, DBA/2J, FVB/N, and SJL/J
- Mice were sacrificed at 3, 5, and 6 months to assess granuloma formation and severity of disease
- Lungs were cut/sectioned and severity of disease was scored

### Severity Score

0 = none

1 = minimal

2 = mild

3 = moderate

4 = marked

5 = severe

### Distribution Score

0 = none

1 = focal

2 = locally extensive

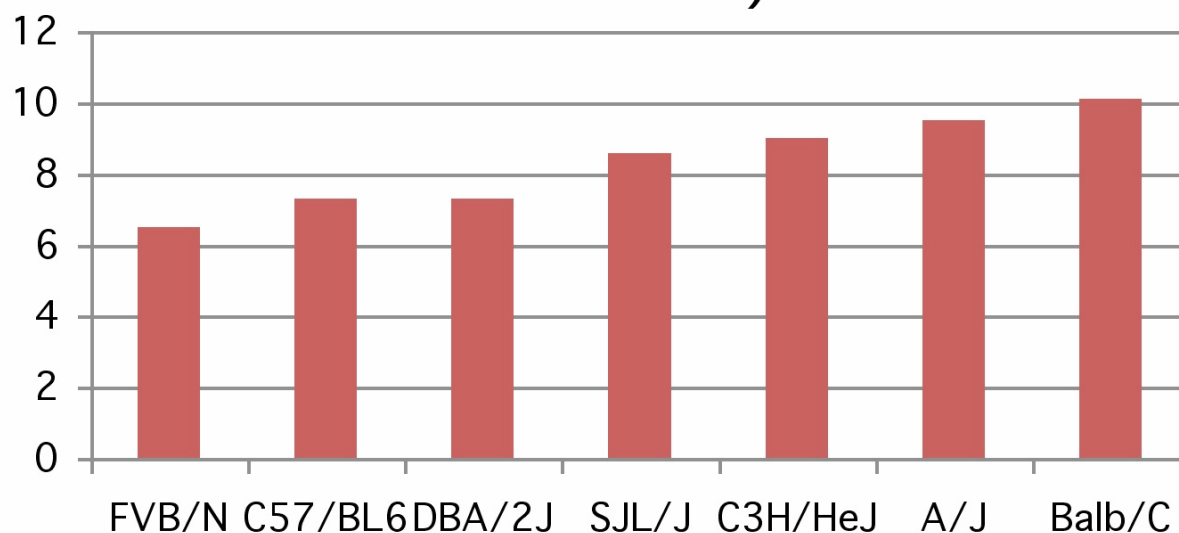
3 = multi-focal

4 = multi-focal coalescent

5 = diffuse

# 5 Month Pilot Aspiration Study (pseudo-area X severity)

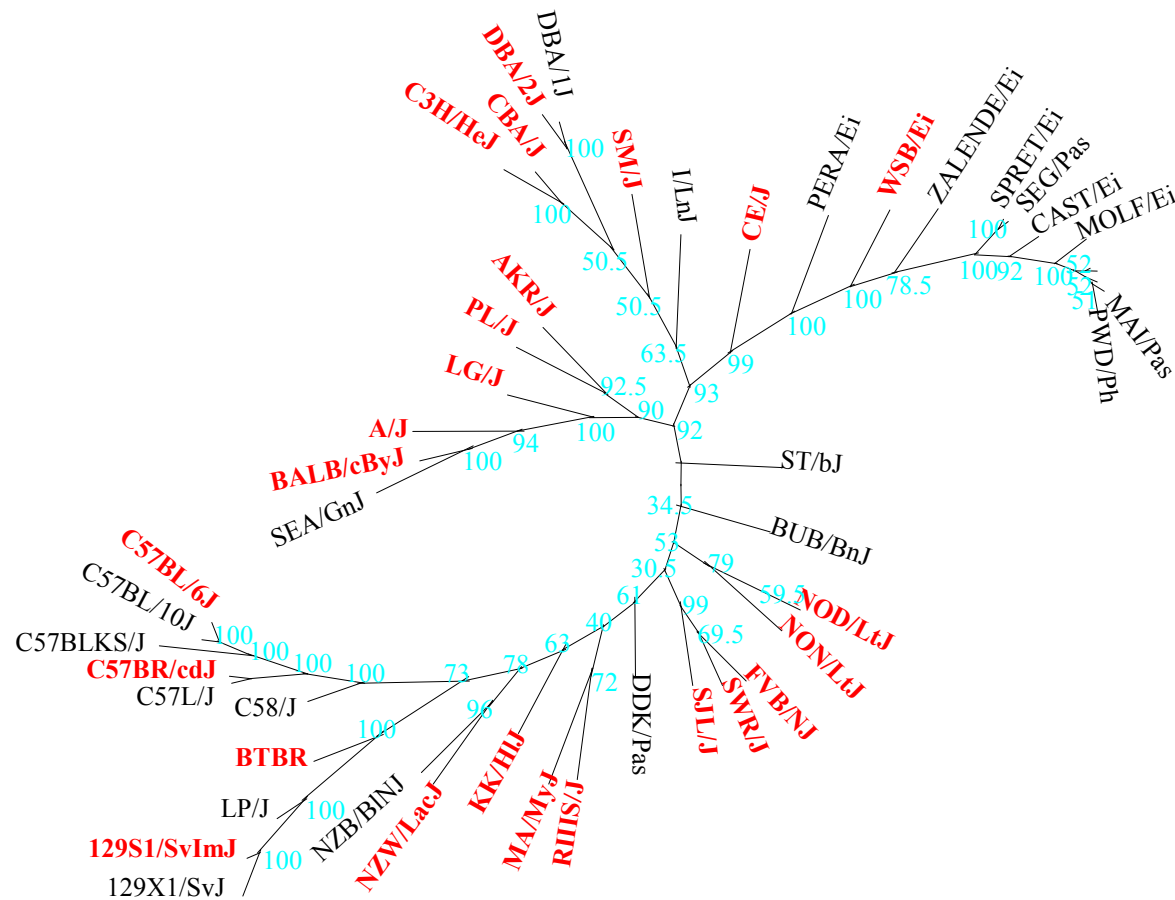
## Granuloma Study (Severity \* Distribution)



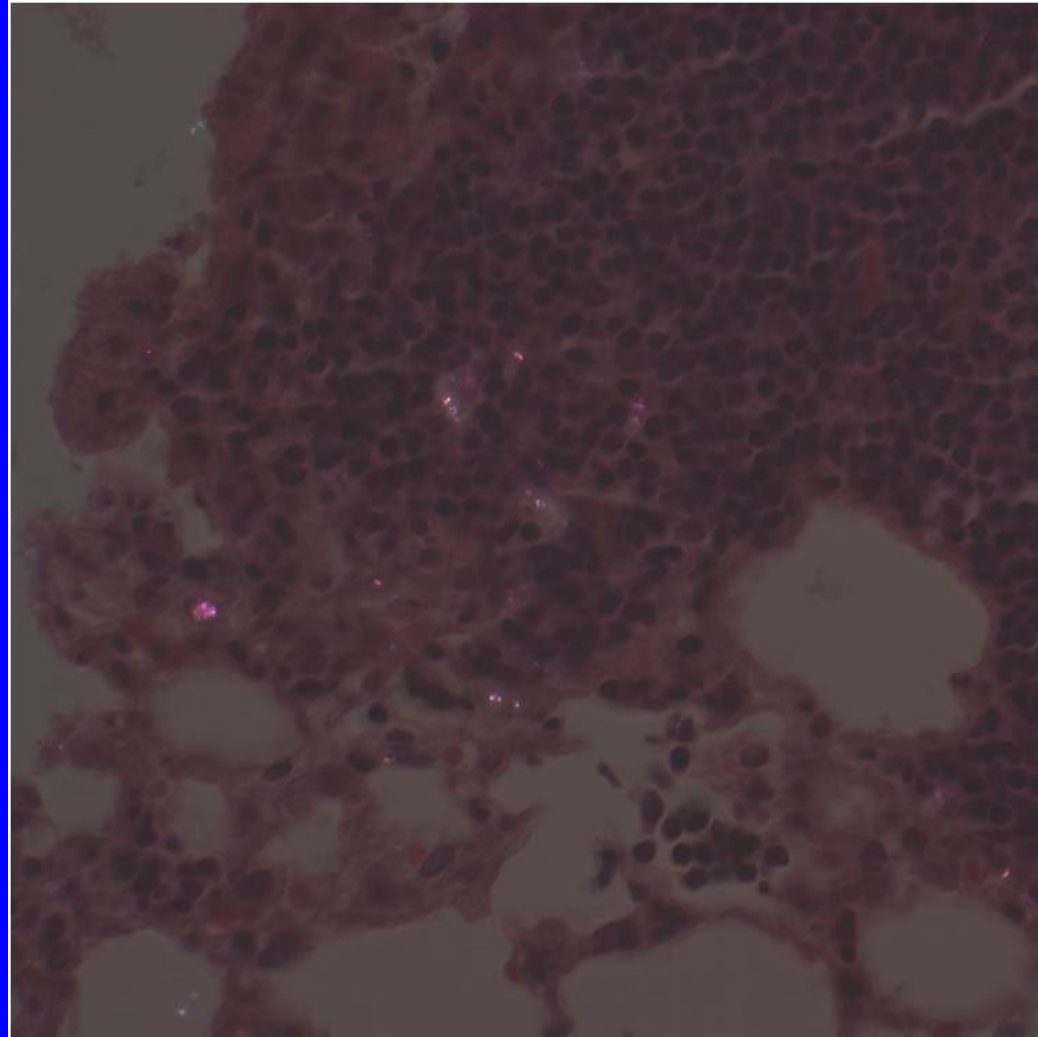
# Expanded Lung Study (Round 4)

- Includes 23 inbred mouse strains
- Dose - 20  $\mu\text{g}$ /month Be
- Generated Disease score based upon:
  - severity of lesion
  - distribution (i.e., focal or dispersed)
  - demarcation

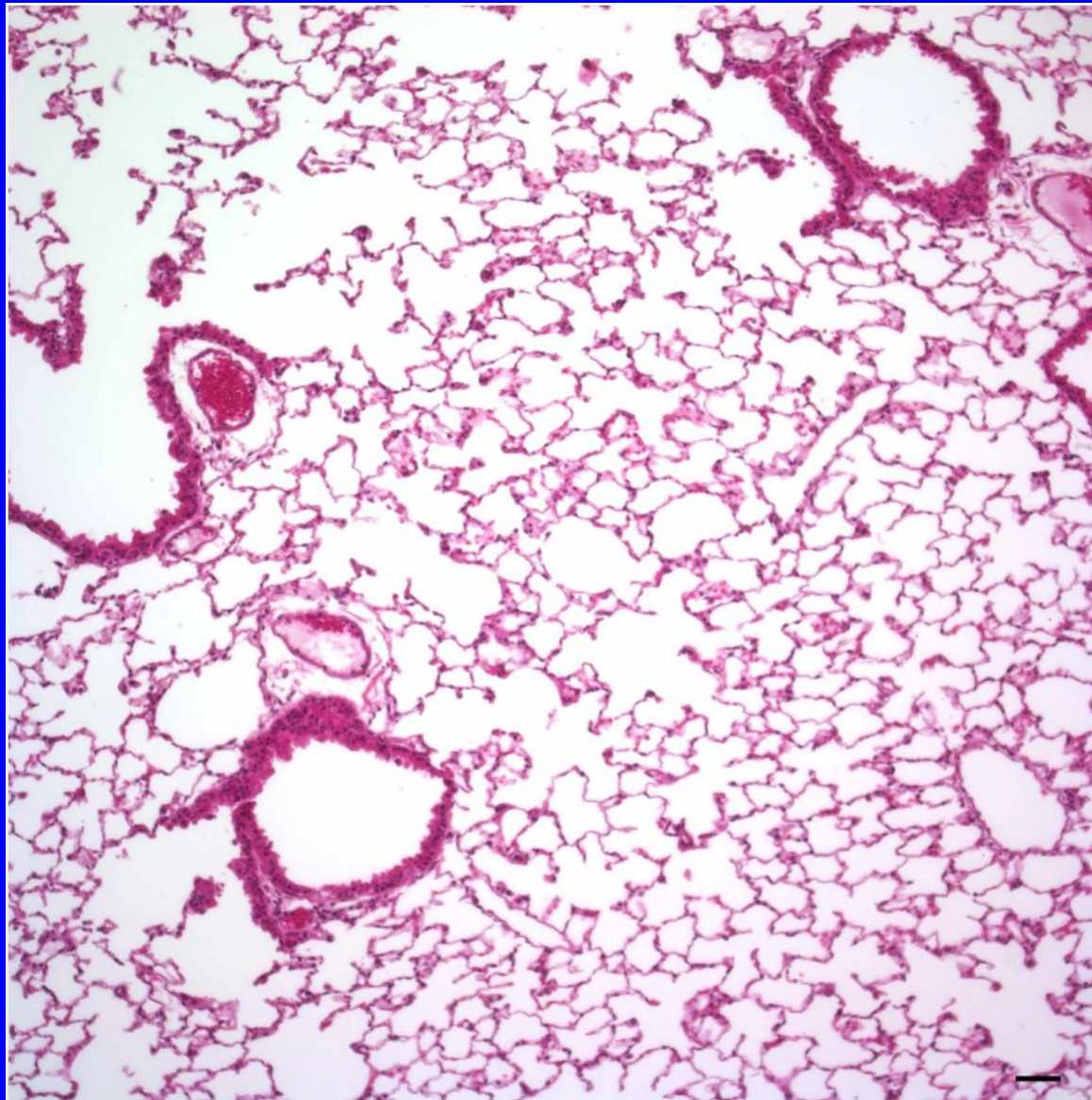
# Expanded 5 Month Aspiration Study



# Histopathology – Granulomas Associated with Beryllium?

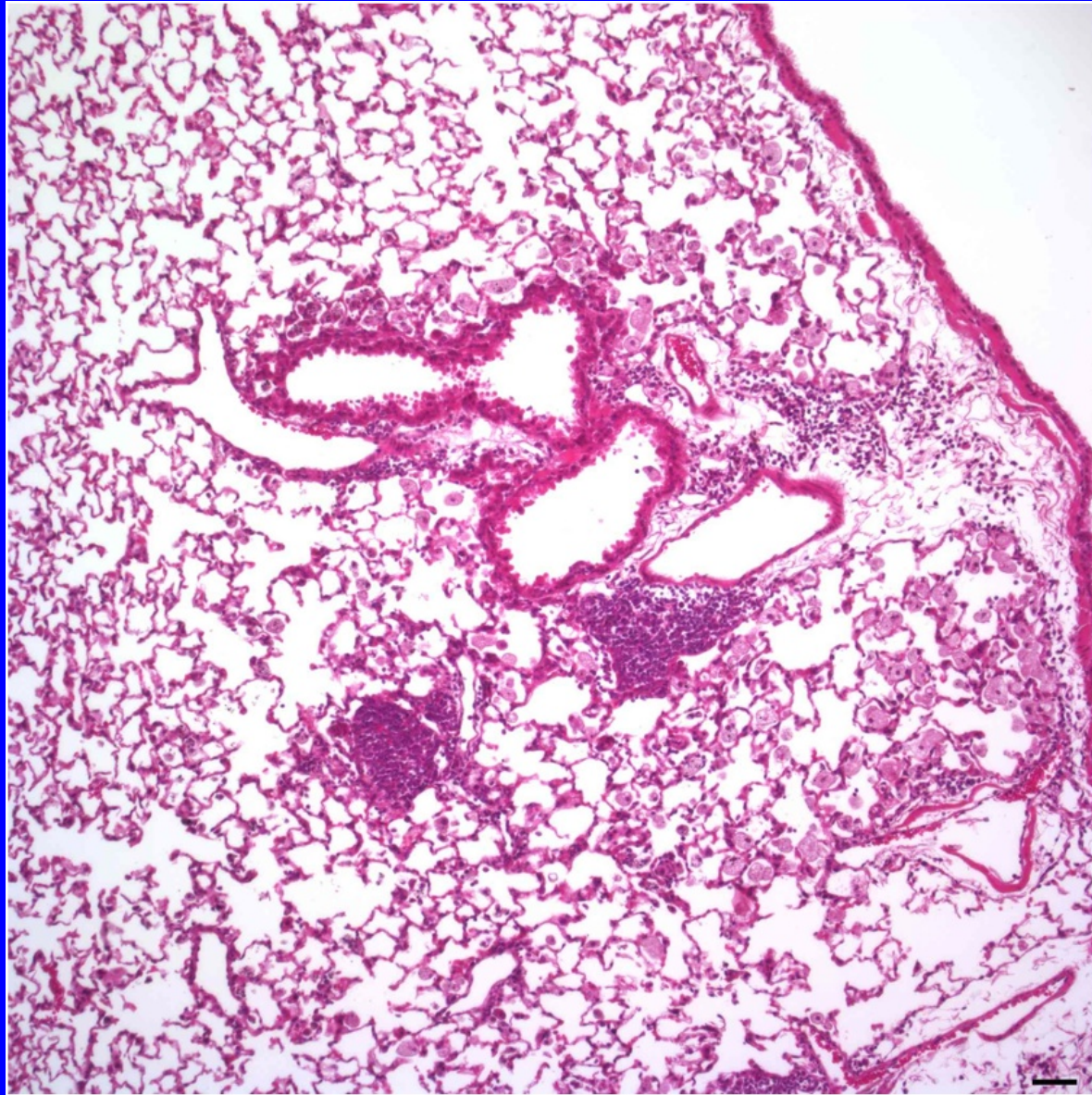


# Histopathology – Non-responsive Strain



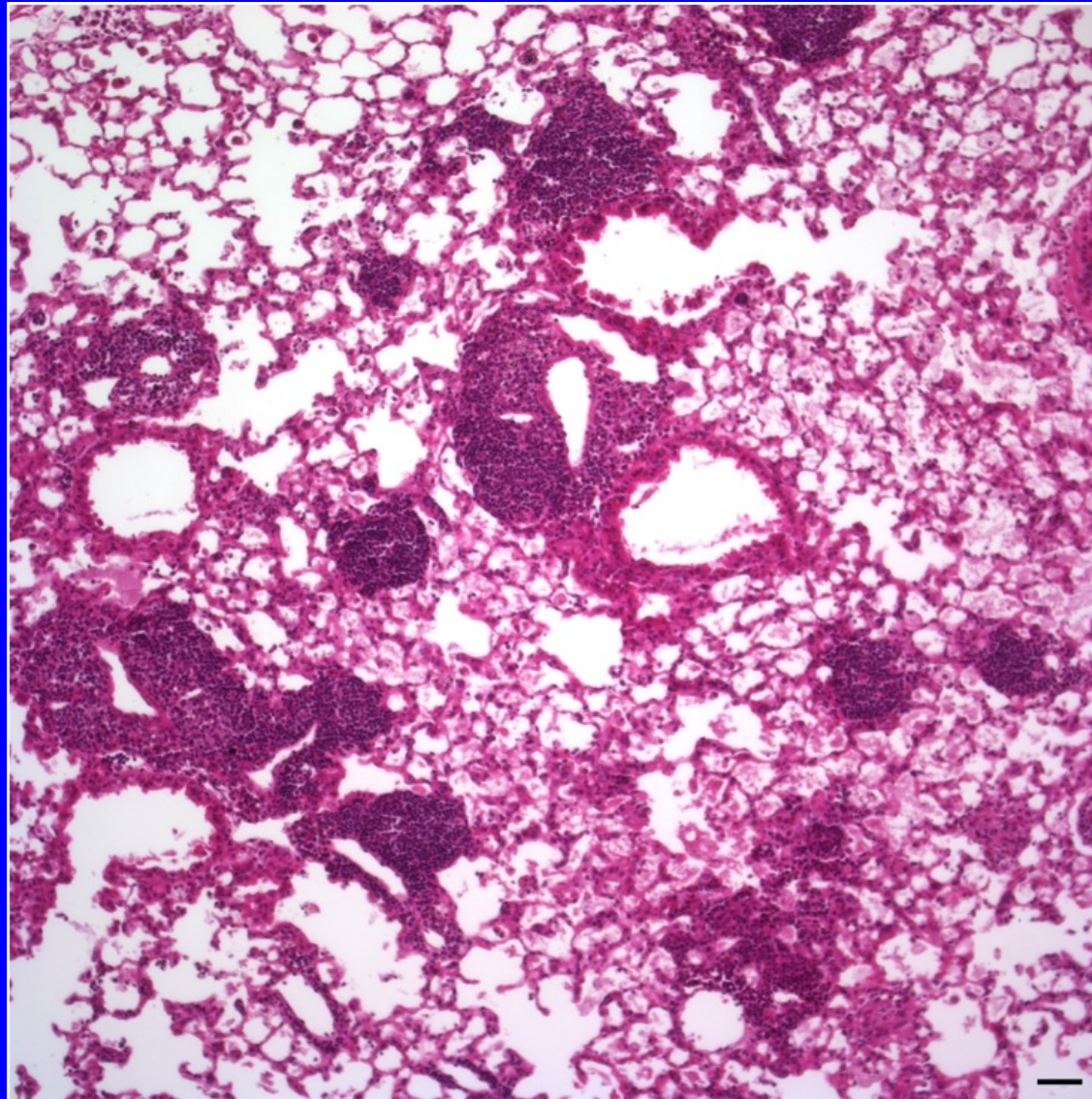


# Histopathology – Responsive Strain



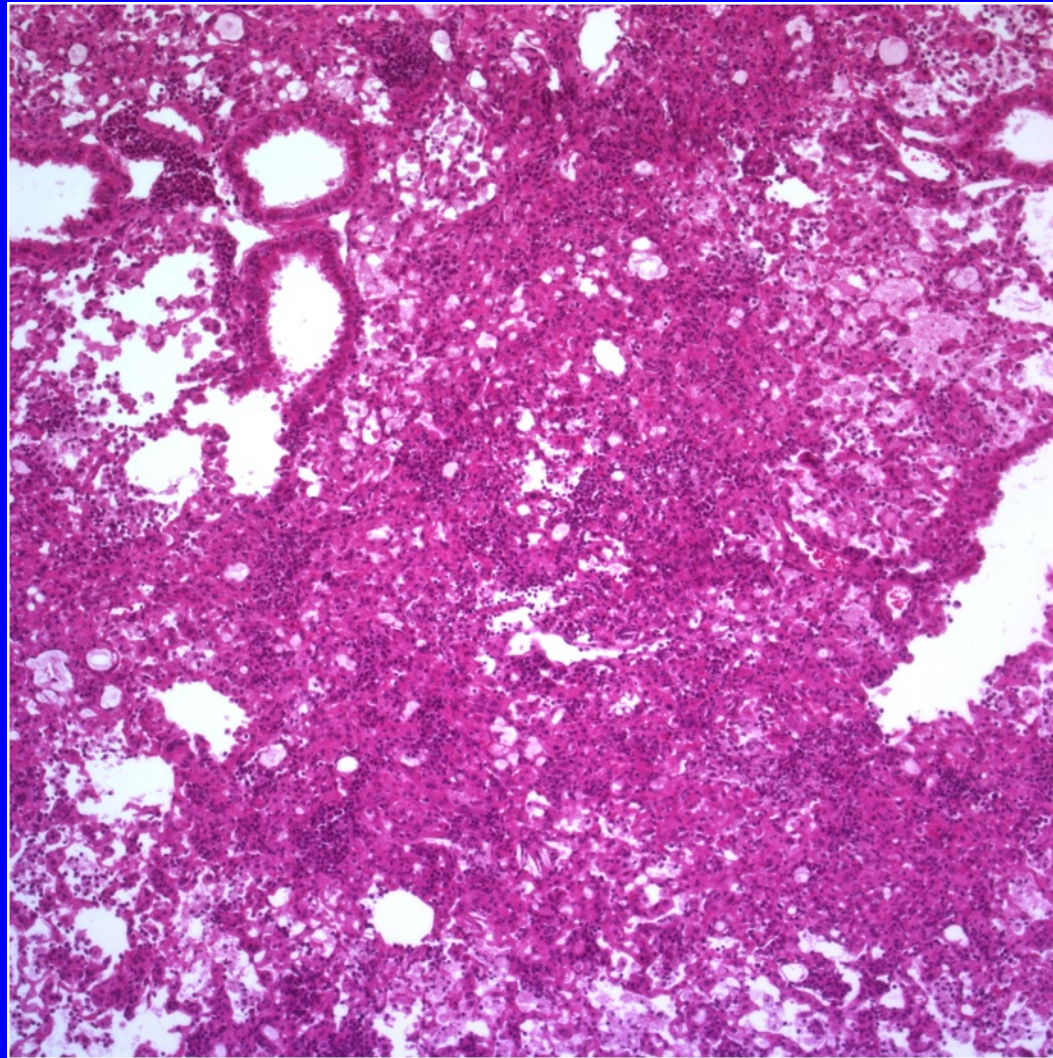


# Histopathology – Responsive Strain

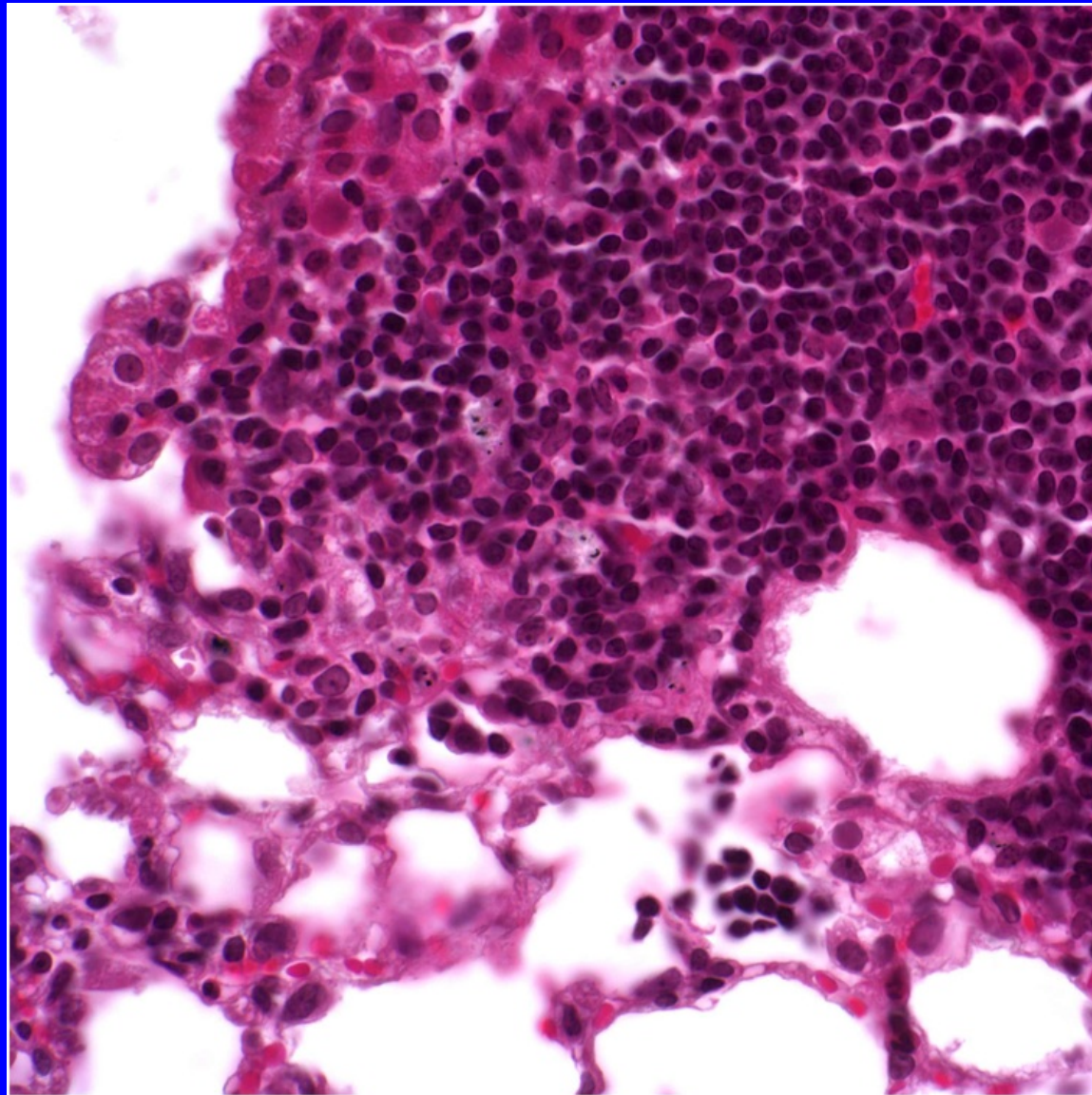




# Histopathology – Responsive Strain

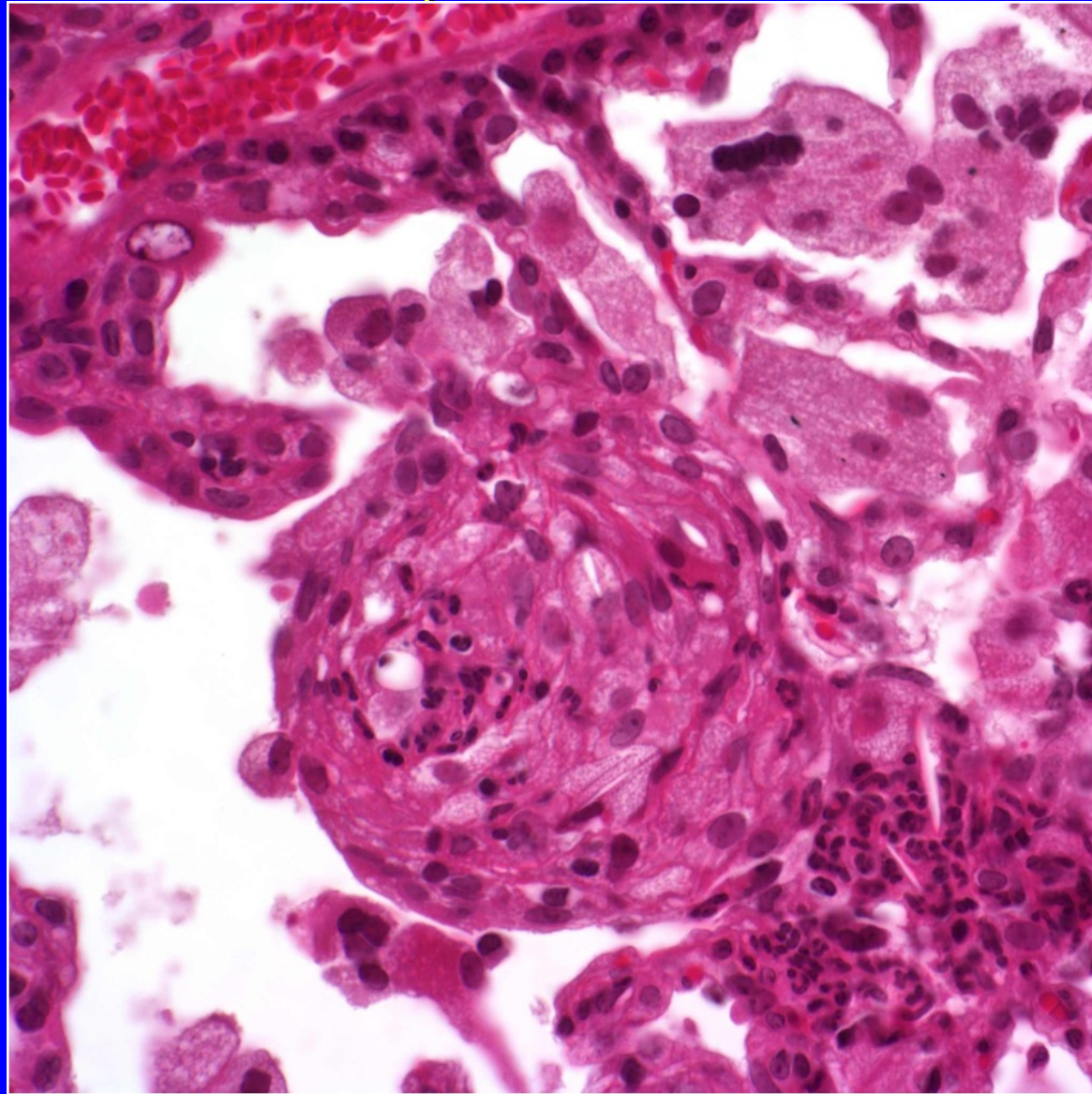


# Histopathology – Responsive Strain





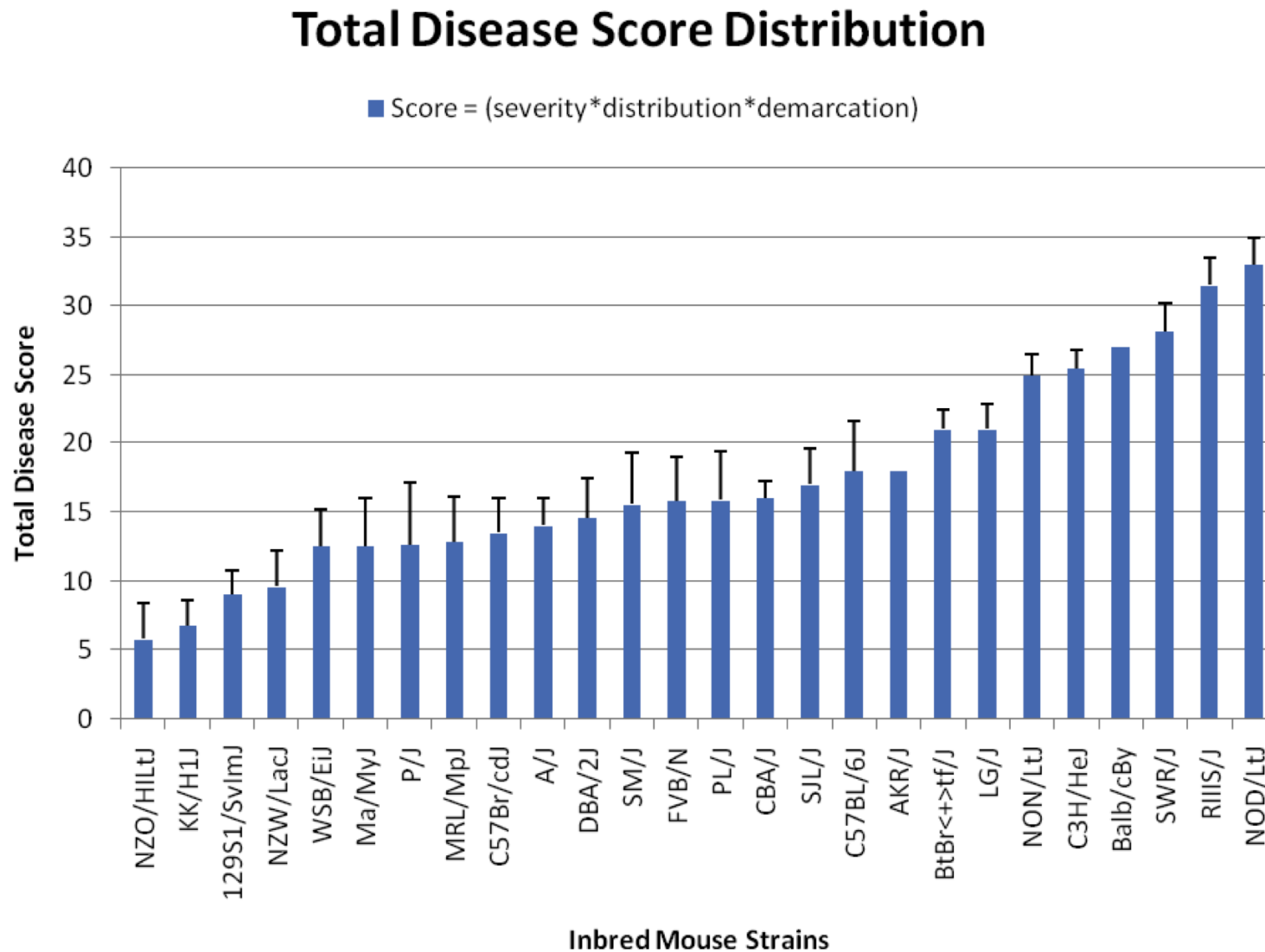
# Histopathology – Caplan's Syndrome?



# Histopathology – Caplan's Syndrome?

- Anuclear macrophages are frequently observed
- Alveolar histiocytosis, lipoproteinosis, epithelial cell hypertrophy and hyperplasia
- Neutrophilic interstitial pneumonia
  - accompanied by cholesterol clefts, with neutrophilic infiltration being highly variable
  - Fibrosis is associated with these foci and the rare fibrohistiocytic nodules have a slightly lamellar organization, reminiscent of Caplan's nodules
- Subpleural fibrosis

# Differential Response in Lung Granulomas (26 Mouse Strains)





# No Overlap in Genes for Sensitization and Granulomas

<b>Lung Granuloma</b>			<b>Skin Sensitizat</b>	
Chromosome	MBases (DNA)		Chromosome	MBases (DNA)
2	29.1-30.3		1	81.5-81.6
4	108.5-108.8		3	44.4-44.7
4	110.7-111.0		5	124.1-124.1
5	61.7-62.04		12	72.2
5	63.3-63.6		18	45.7-45.8
6	7.5-7.7			
15	14.2-14.4			



# Biomarkers of Effect and/or Exposure

- LPT
- Anything better than LPT?





# Biomarkers of Effect and/or Exposure

(DOE support - Drs. Gordon and  
Fontenot)

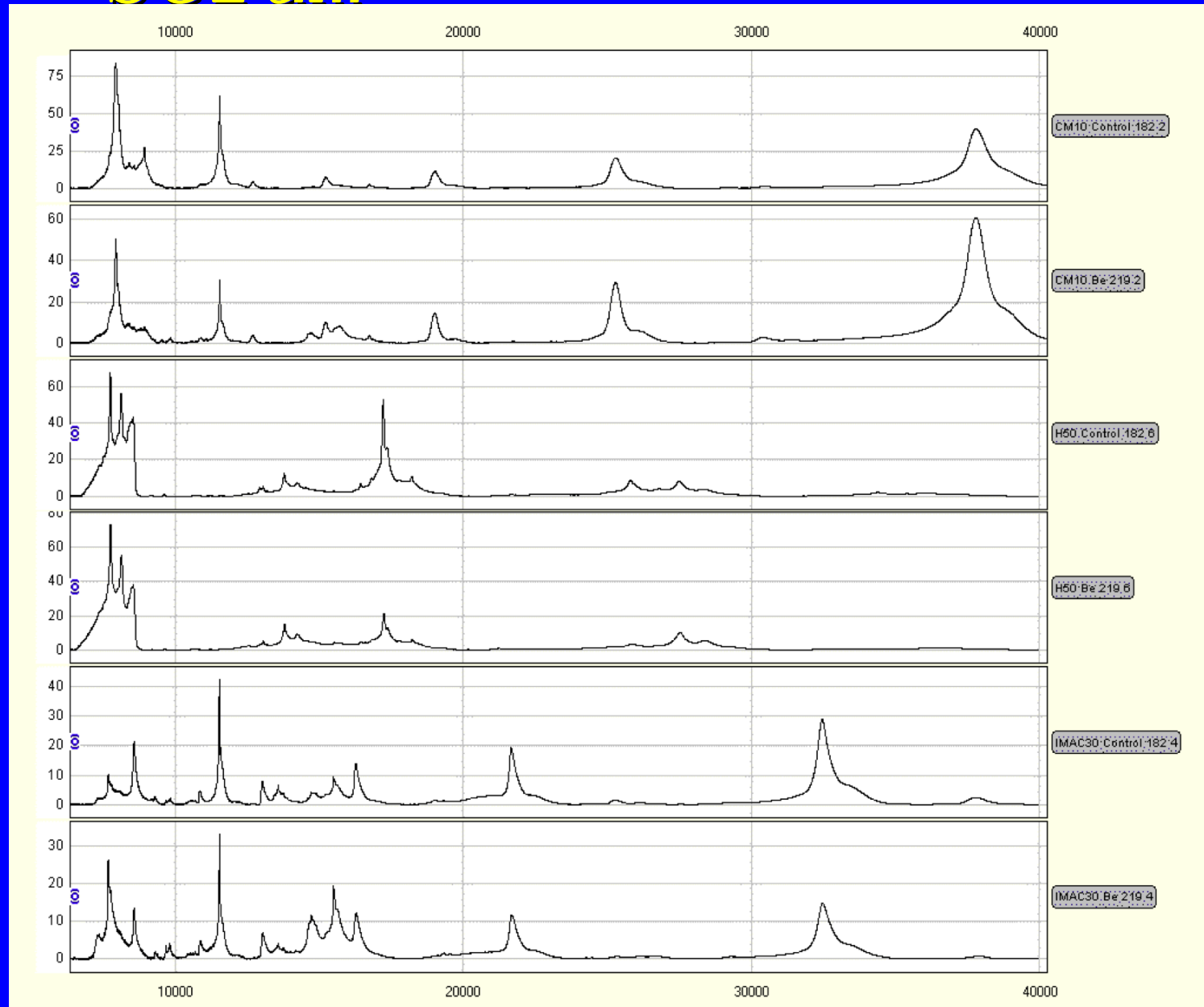
- Lymphocyte  
proliferation
- EliSpot
- Proteomics



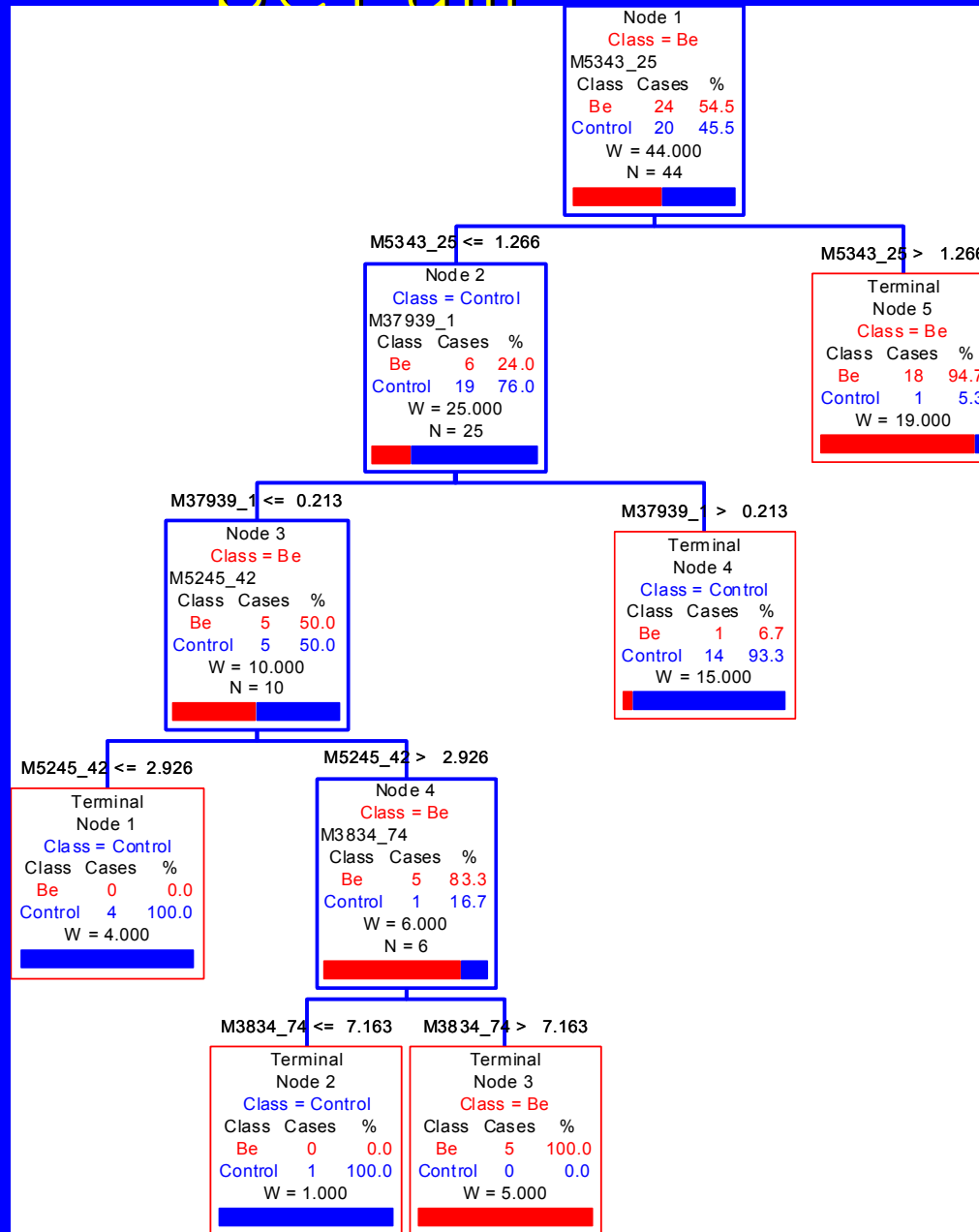
# Proteomics Methods

- Treat mice with beryllium
  - dermal or aspiration
- Sample serum
- Fractionate and analyze proteins by CIPHERGEN's SELDI-TOF mass spectroscopy

# PROTEIN BIOMARKERS IN Serum



# PROTEIN BIOMARKERS IN Serum



CART = Classification  
and regression tree  
analysis

Collaboration w/ Brian  
Tooker and Lee Newman

# Conclusions

- Human transgene \*1701 produced greater skin sensitization response in inbred mice
- Strain differences in the inbred mouse strains seen in both the skin sensitization and the granuloma studies
  - Suggests a contribution of genetic factors
  - Different genes for sensitization vs. granulomas
- A dose/response for granulomas was observed for beryllium metal

# Collaborators

Karen Galdanes

David Delano

Tim Wiltshire

Andrew Fontenot

Lee Newman

Brian Tooker

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